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# Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States

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**Abstract**

Recent studies on sheep and goat farms in the southern United States indicate that multiple-anthelmintic resistance in *Haemonchus contortus* is becoming a severe problem. Though many factors are involved in the evolution of resistance, the proportion of the parasite population under drug selection is believed to be the single most important factor influencing how rapidly resistance develops. Therefore, where prevention of resistance is an important parallel goal of worm control, it is recommended to leave a portion of the animals untreated. Recently, a novel system called FAMACHA© was developed in South Africa, which enables clinical identification of anemic sheep and goats. When *H. contortus* is the primary parasitic pathogen, this system can be applied on

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the farm level to reduce the number of treatments administered, thereby increasing the proportion of the worm population in refugia. Since most studies validating the FAMACHA© method have been performed in South Africa, it is important that the method be tested in other regions before its use is broadly recommended. We performed a validation study of FAMACHA© by testing the system in sheep ($n = 847$) and goats ($n = 537$) of various breeds and ages from 39 farms located in Arkansas, Georgia, Louisiana, Florida, and the US Virgin Islands. The color of the ocular conjunctiva of all animals were scored on a 1–5 scale using the FAMACHA© card, and blood samples were collected from each animal for determination of packed cell volume (PCV). Fecal samples were also collected from a majority of the animals tested for performance of fecal egg counts (FEC). Correlations between PCV and eye scores, PCV and FEC, and FEC and eye scores were all highly significant for both sheep and goats ($P < 0.001$). Data for both FAMACHA© scores and PCV were evaluated using two separate criteria for anemia: eye score values of 3, 4 and 5 or 4 and 5, and PCV values of ≤19 or ≤15 were considered anemic. Specificity was maximized when eye score values of 4 and 5 were considered anemic and PCV cut off for anemia was ≤19, but sensitivity was low. In contrast, sensitivity was 100% for both sheep and goats when eye score values of 3, 4 and 5 were considered anemic and PCV cut off was ≤15, but specificity was low. In both sheep and goats, predictive value of a negative was greater than 92% for all anemia and eye score categories, and was greater than 99% for both eye score categories when an anemia cutoff of ≤15 was used. Predictive value of a positive test was low under all criteria indicating that many non-anemic animals would be treated using this system. However, compared to conventional dosing practices where all animals are treated, a large proportion of animals would still be left untreated. These data indicate that the FAMACHA© method is an extremely useful tool for identifying anemic sheep and goats in the southern US and US Virgin Islands. However, further studies are required to determine optimal strategies for incorporating FAMACHA©-based selective treatment protocols into integrated nematode control programs.


*Keywords:* FAMACHA©; Eye color chart; Sheep; Goat; Anthelmintic resistance; *Haemonchus contortus*

## 1. Introduction

Production of small ruminants is an attractive enterprise for farmers in the southern United States due to the relatively low cost of breeding stock, a high reproductive rate, requirement for minimal capital input as compared to other livestock species, and the ability of small ruminants to thrive on native pasture or brushland that is unsuitable for cropping or grazing by cattle (Glimp, 1995). These factors and a high demand for small ruminant meat and milk products has led to tremendous growth in the size and scope of the United States (US) goat industry over the past 10 years, with most of this growth occurring in the southern region (Getz, 2002 unpublished report). A concurrent trend with increasing numbers of goats in the southern US is the intensification of production systems. Among the many challenges created by the intensification of production systems, control of gastrointestinal nematode (GIN) parasites is the most important. A recent 7-year review of clinical cases at Auburn University Veterinary Medical Teaching Hospital in Alabama found that parasite infection was the primary reason that 70% of sheep and 91% of goats were examined and treated by hospital clinicians (Pugh et al., 1998; Pugh and Navarre, 2001). In a different survey, abomasal or intestinal worm infection was identified as the predominant disease condition present on 74% of sheep farms (USDA, 2003). Although several species of GIN co-infect these animals, *Haemonchus contortus*, a blood-sucking parasite that thrives in warm climates is generally recognized as the single most important pathogen, and on the majority of sheep and goat farms in the southern US, eggs of *H. contortus* usually account for 75–100% of the total fecal nematode egg output (Mortensen et al., 2003; Uhlinger et al., 1992). Infection with *H. contortus* may cause severe anemia and hypoproteinemia, leading to depression, loss of condition, reduced productivity, and eventual death. The disease tends to be more severe in young kids and lambs, but mature animals can also be severely affected.

In this region, efficient production of small ruminants has been made possible by the frequent use of anthelmintics. This practice has permitted rapid growth of the meat goat industry through the 1990’s, but also has resulted in the selection of worm populations that are resistant to these drugs (Miller and Craig, 1996; Mortensen et al., 2003; Terrill et al., 2001; Zajac and Gipson, 2000). Although anthelmintic resistance has been recognized globally as the single greatest threat to small ruminant production since the mid 1990’s (Waller, 1997), until recently this problem has been all but ignored in the US. However, a recent survey of anthelmintic resistance on goat farms in Georgia (southern US) found that 90% of farms had multiple-resistances to both albendazole and ivermectin, and 30% of those same farms also had resistance to levamisole. Furthermore, in a study conducted in Georgia in 2003, moxidectin resistance was found on more than half of all farms with a history of routine use of this drug in the previous 2–3 years (Kaplan, unpublished). These data suggest that anthelmintic resistance in the southern US may be as severe as in other areas of the world such as South America, South Africa and Malaysia, where multiple-anthelmintic resistance has been well documented (Chandrawathani et al., 2003; Echevarria et al., 1996; Maciel et al., 1996; Van Wyk et al., 1999). In these and other warm, humid areas of the world, the inability to control multiple-drug-resistant *H. contortus* seriously threatens the future viability of established small ruminant industries.

The typical strategy used by small ruminant producers for controlling *H. contortus* involves the treatment of all animals at fixed frequent intervals during peak transmission periods and/or treating the entire group when one or more animals demonstrate clinical symptoms suggestive of worm infection. Other nematode control schemes based upon epidemiological principles that have been strongly recommended over the years include suppressive drenching early in the transmission season and treat-and-move strategies (Herd et al., 1984). Although these strategies are very effective in controlling nematode parasites, they also place heavy genetic selection pressure for resistance on nematode populations. This is because all worms in all animals are exposed to the drug, and unexposed environmental refugia (consisting of eggs and larval stages on pasture) are purposely kept at a low level. In contrast to previous epidemiologically-based recommendations designed to maximize parasite control, recommendations must now be designed to not only control parasites, but also to minimize the development of anthelmintic resistance. We now recognize that the proportion of the population under drug selection is the single most important factor influencing how rapidly resistance will develop (Van Wyk et al., 2002). Therefore, nematode control programs should be designed to maintain the maximum amount of refugia (portion of the population not exposed to the drug) that is consistent with acceptable parasite control (Van Wyk, 2001). Maintaining a large refugium causes resistance to develop at a much slower rate because worms in refugia provide a pool of genes that are sensitive to anthelmintics, which dilute the frequency of resistant alleles in the population and reduce the chances of worms carrying resistant alleles from mating with other resistant worms. Such a program will permit efficient production to continue, but will considerably reduce the rate of evolution for resistance and thus preserve the efficacy of the few remaining anthelmintics that are required for life-saving intervention.

In order to accomplish the goal of maximizing refugia, it is necessary to leave a portion of the herd or flock untreated. Studies on host–parasite dynamics consistently find that parasite burdens are aggregated in groups of animals (Crofton, 1971) with a minority of animals harboring the majority of the parasites (Galvani, 2003; Sreter et al., 1994). In sheep and goats it is common for 20–30% of the animals to harbor 70–80% of the worms. Therefore, a selective approach that targets the portion of the herd or flock with high worm burdens will successfully control parasites in the entire group, while also reducing drug costs and delaying the development of anthelmintic resistance (Barger, 1985).

The major limitation to instituting a selective treatment approach has been the lack of an efficient and economical means of identifying those animals requiring treatment. This problem has recently been solved by a novel system developed in South Africa for identifying sheep that are anemic (Bath et al., 1996). Although this method was developed specifically to address infection with *H. contortus*, which is the most common cause of anemia in small ruminants, it should be noted that there are other parasitic and non-parasitic causes of anemia. In this method, called FAMACHA©, the ocular mucous membranes of sheep and goats are classified by comparison with a laminated color chart bearing pictures of sheep conjunctivae classified into five categories ranging from the normal red, through pink to practically white in severe anemia. Since anemia is the primary pathologic effect from infection with *H. contortus*, this system can be an effective tool for identifying those animals that require treatment (but only for *H. contortus*). FAMACHA© has been extensively tested in South Africa with excellent results. In an early clinical evaluation trial where animals were examined at weekly intervals and salvage treatments were only administered if packed (red blood) cell volume (PCV) was 15% or less, only 10% of the flock required more than one salvage treatment and 70% of adult sheep did not require any treatment (Malan et al., 2001). Compared to the previous treatment regimen used on this farm, the total number of treatments given decreased by approximately 90%, but it should be kept in mind that this constituted a relatively severe level of worm challenge. In subsequent studies where FAMACHA© was used by farmers and treatments were based solely on FAMACHA© scores without the aid of PCV determinations, mean reductions on 10 farms in the number of treatments from previous years was still 58% (Van Wyk and Bath, 2002). FAMACHA© has also been validated on goat farms in South Africa (Vatta et al., 2001). This technique appears to be less accurate in goats than in sheep, but still provides a good tool for implementing a selective treatment approach. Because all published studies to date validating the FAMACHA© method have been performed in South Africa, it is important that the method be tested in other areas of the world before its use can be broadly recommended. The purpose of the present study was to validate the FAMACHA© method on sheep and goat farms in the southern US and the Caribbean.

## 2. Materials and methods

### 2.1. Animals and procedures

Weaned and mature sheep ($n = 847$) and goats ($n = 537$) of various breeds and ages from 39 farms located in Arkansas (sheep, $n = 370$; goats, $n = 148$), Georgia (sheep, $n = 167$; goats, $n = 170$), Louisiana (sheep, $n = 107$; goats, $n = 78$), Florida (sheep, $n = 72$; goats, $n = 21$), and the Virgin Islands (sheep, $n = 131$; goats, $n = 120$) were evaluated in this study during the periods June through December 2002 and March through May 2003. The minimum, maximum, mean, and median number of animals examined on a farm were 14, 151, 36, and 25, respectively. The color of the ocular mucous membranes of each animal was examined on one occasion and classified into one of five categories according to the FAMACHA© eye color chart: 1 = red, non-anemic; 2 = red-pink, non-anemic; 3 = pink, mildly-anemic; 4 = pink-white, anemic; 5 = white, severely anemic. All animals were scored by the co-investigator(s) located in each state where animals were evaluated. On farms where more than one investigator scored animals, the average of the two scores was used in the analysis. Prior to initiating this study, each co-investigator received training in this method from Dr. A.F. Vatta, who is an experienced practitioner of FAMACHA©. Blood samples were collected from each animal for determination of packed cell volume (PCV) and feces were collected from a majority of the animals for determination of fecal nematode egg counts (FEC) using a modified McMaster's technique (Whitlock, 1948) with a sensitivity of 50 eggs per gram (EPG) of feces. On 27 farms, cultures of nematode larvae were prepared from a pooled fecal sample. Infective third-stage larvae ($L_3$) were recovered and identified to genus (M.A.F.F., 1977).

All experimental procedures were reviewed and accepted by the Animal Care and Use Committee of each institution. Pain and stress to animals were minimized throughout the experimental period.

### 2.2. Statistical analysis

Data from sheep and goats were analyzed separately. Spearman correlation coefficients were calculated for each species using SAS (1996) to examine the relationship between eye scores, PCV and FEC. Arithmetic means and standard errors of FEC were calculated. Box and whisker plots were drawn to represent the distribution of PCV in relation to FAMACHA© scores.

Two-way frequency tables with PCV by eye score were created according to Vatta et al. (2001). Eye score values of 3, 4 and 5 or 4 and 5 were considered anemic and eye score values of 1 and 2 or 1, 2 and 3 were considered non-anemic. Packed cell volume values were considered anemic if $\leq$19 or $\leq$15. These two levels were used to provide alternative views of the data; since no precise value for PCV has been clearly established at which anemia crosses a threshold of clinical importance. Sensitivity, specificity, predictive value of a negative, and predictive value of a positive were calculated for the data according to Vatta et al. (2001). A true positive result was defined as animals that were anemic (PCV $\leq$15 or $\leq$19%) with pale eye scores (4, 5 or 3, 4, 5). A false positive result was defined as animals that were not anemic (PCV >15 or >19%) with pale eye scores. A false negative result was defined as animals that were anemic with red or pink eye scores (1, 2 or 1, 2, 3). A true negative result was defined as animals that were not anemic with pink or red eye scores (Vatta et al., 2001).

## 3. Results

Based on results of fecal cultures, *H. contortus* was the primary gastrointestinal nematode parasite infecting animals on all 27 farms where cultures were performed. The overall mean for percent of *H. contortus* $L_3$ recovered from feces on the 27 sheep and goats farms was 91%. *Trichostrongylus* spp. was the next most common genus averaging 9% across all farms. Occasional larvae of *Cooperia* spp. and *Oesophagostomum* spp. were also present.

Correlations between PCV and eye scores, PCV and FEC, and FEC and eye scores were all highly significant for both sheep and goats ($P < 0.001$). Correlations were negative between PCV and eye scores (sheep: $R = -0.52$; goats: $R = -0.30$) and PCV and FEC (sheep: $R = -0.49$; goats: $R = -0.50$), and were positive between FEC and eye scores (sheep: $R = 0.21$; goats: $R = 0.29$). Means and standard errors of FEC were calculated for sheep and goats in each FAMACHA© score category (Table 1).

FAMACHA© eye score values were compared with percent PCV for sheep (Tables 2–5) and goats (Tables 6–9) to determine rates of false negatives, false positives and correct treatment decisions as defined by the parameters established for anemia and need for treatment. Sixty-two and 89% of recommendations to deworm were correct when treating sheep

Table 1
Mean and standard error (S.E.) of fecal egg counts (eggs per gram) by FAMACHA© eye score category for sheep and goats

| FAMACHA© score | Sheep | | Goats | |
|---|---|---|---|---|
| | *n* | Mean (S.E.) | *n* | Mean (S.E.) |
| 1 | 93 | 514 (100) | 31 | 266 (47) |
| 2 | 215 | 716 (168) | 78 | 645 (88) |
| 3 | 214 | 1195 (189) | 93 | 1372 (222) |
| 4 | 76 | 2007 (524) | 82 | 1451 (395) |
| 5 | 12 | 7762 (1796) | 10 | 3845 (698) |

Table 2
Sheep: frequency and percent (in parenthesis) of false negatives, false positives and correct treatment recommendations for assigned ranges in PCV values based on treatment of sheep with FAMACHA© eye scores of 3, 4 and 5

| PCV value | False negative | False positive | Correct treatment | Total |
|---|---|---|---|---|
| ≤19 | 5 (0.6) | – | 59 (7.0) | 64 (7.6) |
| 20–29 | – | 235 (27.7) | 181 (21.4) | 416 (49.1) |
| >29 | – | 84 (9.9) | 283 (33.4) | 367 (43.3) |
| Total | 5 (0.6) | 319 (37.6) | 523 (61.8) | 847 (100) |

Incorrect treatment would have occurred if eye score was 3, 4 or 5 and PCV >19 (false positive) and if eye score was 1 or 2 and PCV ≤19 (false negative).

Table 3
Sheep: frequency and percent (in parenthesis) of false negatives, false positives and correct treatment recommendations for assigned ranges in PCV values based on treatment of sheep with FAMACHA© eye scores of 4 and 5

| PCV value | False negative | False positive | Correct treatment | Total |
|---|---|---|---|---|
| ≤19 | 23 (2.7) | – | 41 (4.8) | 64 (7.6) |
| 20–29 | – | 59 (7.0) | 357 (42.1) | 416 (49.1) |
| >29 | – | 9 (1.1) | 358 (42.3) | 367 (43.3) |
| Total | 23 (2.7) | 68 (8.1) | 756 (89.3) | 847 (100) |

Incorrect treatment would have occurred if eye score was 4 or 5 and PCV >19 (false positive) and if eye score was 1, 2 or 3 and PCV ≤19 (false negative).

Table 4
Sheep: frequency and percent (in parenthesis) of false negatives, false positives and correct treatment recommendations for assigned ranges in PCV values based on treatment of sheep with FAMACHA© eye scores of 3, 4 and 5

| PCV value | False negative | False positive | Correct treatment | Total |
|---|---|---|---|---|
| ≤15 | 0 (0) | – | 23 (2.7) | 23 (2.7) |
| 16–29 | – | 271 (32.0) | 186 (22.0) | 457 (54.0) |
| >29 | – | 84 (9.9) | 283 (33.4) | 367 (43.3) |
| Total | 0 (0) | 355 (41.9) | 492 (58.1) | 847 (100) |

Incorrect treatment would have occurred if eye score was 3, 4 or 5 and PCV >15 (false positive) and if eye score was 1 or 2 and PCV ≤15 (false negative).

Table 5
Sheep: frequency and percent (in parenthesis) of false negatives, false positives and correct treatment recommendations for assigned ranges in PCV values based on treatment of sheep with FAMACHA© eye scores of 4 and 5

| PCV value | False negative | False positive | Correct treatment | Total |
|---|---|---|---|---|
| ≤15 | 4 (0.5) | – | 19 (2.2) | 23 (2.7) |
| 16–29 | – | 81 (9.6) | 376 (44.4) | 457 (54.0) |
| >29 | – | 9 (1.1) | 358 (42.3) | 367 (43.3) |
| Total | 4 (0.5) | 90 (10.7) | 753 (88.9) | 847 (100) |

Incorrect treatment would have occurred if eye score was 4 or 5 and PCV >15 (false positive) and if eye score was 1, 2 or 3 and PCV ≤15 (false negative).

Table 6
Goats: frequency and percent (in parenthesis) of false negatives, false positives and correct treatment recommendations for assigned ranges in PCV values based on treatment of goats with FAMACHA© eye scores of 3, 4 and 5

| PCV value | False negative | False positive | Correct treatment | Total |
|---|---|---|---|---|
| ≤19 | 4 (0.7) | – | 62 (11.6) | 66 (12.3) |
| 20–29 | – | 198 (36.9) | 94 (17.5) | 292 (54.4) |
| >29 | – | 106 (19.7) | 73 (13.6) | 179 (33.3) |
| Total | 4 (0.7) | 304 (56.7) | 229 (42.6) | 537 (100) |

Incorrect treatment would have occurred if eye score was 3, 4 or 5 and PCV >19 (false positive) and if eye score was 1 or 2 and PCV ≤19 (false negative).

Table 7
Goats: frequency and percent (in parenthesis) of false negatives, false positives and correct treatment recommendations for assigned ranges in PCV values based on treatment of goats with FAMACHA© eye scores of 4 and 5

| PCV value | False negative | False positive | Correct treatment | Total |
|---|---|---|---|---|
| ≤19 | 28 (5.2) | – | 38 (7.1) | 66 (12.3) |
| 20–29 | – | 91 (17.0) | 201 (37.4) | 292 (54.4) |
| >29 | – | 37 (6.9) | 142 (26.4) | 179 (33.3) |
| Total | 28 (5.2) | 128 (23.9) | 381 (71.0) | 537 (100) |

Incorrect treatment would have occurred if eye score was 4 or 5 and PCV >19 (false positive) and if eye score was 1, 2 or 3 and PCV ≤19 (false negative).

Table 8
Goats: frequency and percent (in parenthesis) of false negatives, false positives and correct treatment recommendations for assigned ranges in PCV values based on treatment of goats with FAMACHA© eye scores of 3, 4 and 5

| PCV value | False negative | False positive | Correct treatment | Total |
|---|---|---|---|---|
| ≤15 | 0 (0) | – | 18 (3.3) | 18 (3.3) |
| 16–29 | – | 242 (45.1) | 98 (18.2) | 340 (63.3) |
| >29 | – | 106 (19.7) | 73 (13.6) | 179 (33.3) |
| Total | 0 (0) | 348 (64.8) | 189 (35.2) | 537 (100) |

Incorrect treatment would have occurred if eye score was 3, 4 or 5 and PCV >15 (false positive) and if eye score was 1 or 2 and PCV ≤15 (false negative).

with eye scores of 3, 4 and 5 or 4 and 5, respectively, when a PCV value of 19 or less was considered anemic (Tables 2 and 3). Percentage correct was similar when a PCV value of 15 or less was considered anemic (Tables 4 and 5). Using the most conservative guidelines for need and assignment of treatment (PCV ≤19, treatment of sheep in categories 4 and 5) less than 3% of anemic sheep would have failed to receive a required treatment (false negatives; Table 3). However, when more liberal criteria were used, in all cases, less than 1% of anemic sheep would have failed to receive a required treatment (Tables 2, 4 and 5).

When using the same criteria for goats, 43 and 71% of goats would have been correctly treated with eye scores of 3, 4 and 5 or 4 and 5, respectively, when a PCV value of 19 or

Table 9
Goats: frequency and percent (in parenthesis) of false negatives, false positives and correct treatment recommendations for assigned ranges in PCV values based on treatment of goats with FAMACHA© eye scores of 4 and 5

| PCV value | False negative | False positive | Correct treatment | Total |
|---|---|---|---|---|
| ≤15 | 3 (0.6) | – | 15 (2.8) | 18 (3.3) |
| 16–29 | – | 114 (21.2) | 226 (42.1) | 340 (63.3) |
| >29 | – | 37 (6.9) | 142 (26.4) | 179 (33.3) |
| Total | 3 (0.6) | 151 (28.1) | 383 (71.3) | 537 (100) |

Incorrect treatment would have occurred if eye score was 4 or 5 and PCV >15 (false positive) and if eye score was 1, 2 or 3 and PCV ≤15 (false negative).

less was considered anemic (Tables 6 and 7). Using the most conservative guidelines for treatment (PCV ≤19 and eye scores of 4 and 5), less than 6% of goats would have missed necessary treatment. When more liberal criteria were used, in all cases less than 1% of anemic goats would have failed to receive a required treatment (Tables 6, 8 and 9). Using the most liberal criteria (PCV ≤15 and eye scores of 3, 4 and 5), only 35% would have been correctly treated due to a high false positive rate, but none would have missed a necessary treatment (Table 8).

The percentage of sheep recommended for treatment decreased from 44.6% for eye scores of 3, 4 and 5 to 12.9% for eye scores of 4 and 5 [(true positive + false positive)/total number of sheep × 100]. A similar trend was seen with goats, but percentage of goats requiring treatment was higher. Recommending treatment of goats with eye scores of 3, 4 and 5 gave a value of 68.2% of the total and this decreased to 30.9% for goats with eye scores of 4 and 5. For both sheep and goats, sensitivity was maximized when eye score values of 3, 4 and 5 were considered anemic and PCV cut off was ≤15 (Table 10). Specificity was maximized when eye score values of 4 and 5 were considered anemic and PCV cut off was ≤19 (Table 10). In both sheep and goats, predictive value of a negative was greater than 92% for all anemia and eye score categories, and was greater than 99% for both eye score categories when an anemia cutoff of ≤15 was used. However, because of a large number of false positives, the predictive value of a positive was low for all categories. Box and whisker plots showing the relationship between PCV and eye score categories are presented for sheep and goats (Fig. 1).

Table 10
Comparison of sensitivity, specificity, and predictive values for positive and negative tests in sheep and goats using differing FAMACHA© and packed cell volume (PCV) criteria for positive test results and anemia

| | Sensitivity[a] | Specificity[b] | $(a + b)/2 \times 100$ | $PV_{neg}$[c] | $PV_{pos}$[d] |
|---|---|---|---|---|---|
| Sheep | | | | | |
| FAMACHA© values 3, 4, 5 considered positive test results | | | | | |
| PCV cut off ≤19% | 92.2 | 59.2 | 75.7 | 98.9 | 15.6 |
| PCV cut off ≤15% | 100 | 56.9 | 78.5 | 100 | 6.1 |
| FAMACHA© values 4, 5 considered positive test results | | | | | |
| PCV cut off ≤19% | 64.1 | 91.3 | 77.7 | 96.9 | 37.6 |
| PCV cut off ≤15% | 82.6 | 89.1 | 85.8 | 99.5 | 17.4 |
| Goats | | | | | |
| FAMACHA© values 3, 4, 5 considered positive test results | | | | | |
| PCV cut off ≤19% | 93.9 | 35.5 | 64.7 | 97.7 | 16.9 |
| PCV cut off ≤15% | 100 | 32.9 | 66.5 | 100 | 4.9 |
| FAMACHA© values 4, 5 considered positive test results | | | | | |
| PCV cut off ≤19% | 57.6 | 72.8 | 65.2 | 92.5 | 22.9 |
| PCV cut off ≤15% | 83.3 | 70.9 | 77.1 | 99.2 | 9.0 |

[a] Sensitivity = (true positives/(true positives + false negatives)) × 100.
[b] Specificity = (true negatives/(true negatives + false positives)) × 100.
[c] Predictive value of a negative ($PV_{neg}$) = (true negatives/(true negatives + false negatives)) × 100.
[d] Predictive value of a positive ($PV_{pos}$) = (true positives/(true positives + false positives)) × 100.

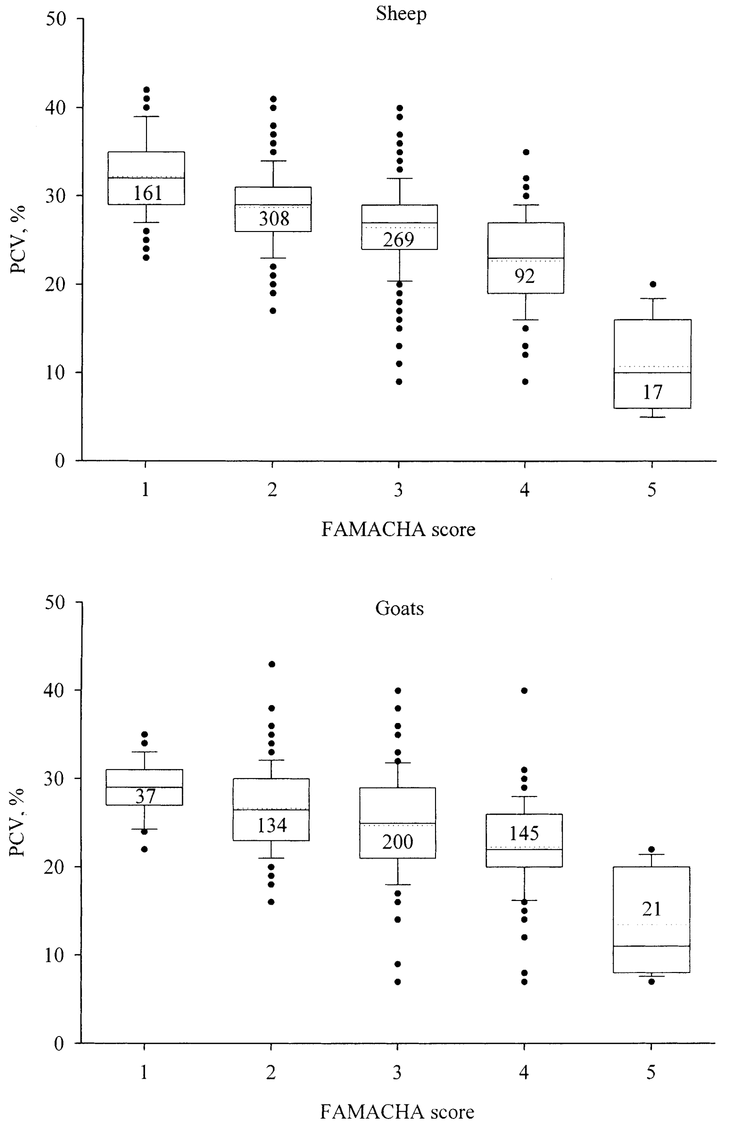


Fig. 1. Box and whisker plots demonstrating the relationship between PCV value and FAMACHA© eye score category in sheep (top panel) and goats (bottom panel). Lower and upper borders of the box represent the 25th and 75th percentiles, respectively. Mean (dotted line) and median (solid line) values are presented within the box. Whiskers above and below the box indicate the 90th and 10th percentiles and the circles represent individual values outside of this range. Values within each box represent the number of animals that were scored within a particular FAMACHA© category.

## 4. Discussion

Indices typically used to measure the accuracy of diagnostic tests include sensitivity, specificity, and predictive values of positive and negative tests (Gerstman and Cappucci, 1986). Critical to establishing the sensitivity and specificity of a diagnostic test are the decision criteria selected for making a positive diagnosis. These decision criteria can be thought of as the threshold amount of evidence favoring the positive event (presence of disease) that is required to issue a positive diagnosis (Swets, 1988). In choosing decision criteria, it is important to consider: (1) the probability of a positive event; and (2) the benefits ascribed to a correct outcome (diagnosis) and the costs ascribed to an incorrect outcome. When the costs of an incorrect outcome (false negative) are high, lenient (liberal) decision criteria are usually preferred. But using lenient decision criteria will result in a positive diagnosis being made relatively often and proportions of both true and false positives will be high causing both reduced specificity and reduced predictive values for positive tests (Swets, 1988). These issues become quite important when evaluating a test such as FAMACHA© because not treating a false negative may mean that an animal dies, but it is quite acceptable to treat a false positive (non-anemic) animal.

In evaluating the ability of FAMACHA© to correctly identify anemic animals in need of treatment, the cut-off for PCV used for declaring anemia will have a great impact on the results. We therefore decided to evaluate our results using two different cut-off values: PCV $\leq$19 and PCV $\leq$15. The higher level was selected because the normal range for PCV in goats is 19–38 (Jain, 1986) and in epidemiologic studies, a PCV of 19 or less is sometimes used as an indication of anemia and the need for salvage treatment. But in reality, an animal with a PCV of 19 is not in any immediate health danger unless pasture conditions of severe *H. contortus* challenge exist. Based upon ranges selected for PCV in sheep scored by FAMACHA©, an animal with a PCV of 18–22 would be scored as a 3, whereas an animal with a PCV of 13–17 would be scored as a 4 (Vatta et al., 2001). Furthermore, initial investigations into the feasibility of using ocular mucous membrane color as a measure for clinical anemia in sheep successfully used a PCV of 15 as a cutoff for administering treatment (Malan et al., 2001). Consequently, a PCV of 19 may be too cautious a cutoff to assess fairly the accuracy of FAMACHA©, and a PCV of 15 may be a more appropriate level to apply. Using liberal criteria for treatment (eye score of 3, 4 or 5) and a definition of anemia of PCV $\leq$15, sensitivity in both sheep and goats was 100% (Tables 4, 8 and 10). This means that using these criteria, every animal scored with the FAMACHA© eye chart that was truly anemic and in need of treatment would have received treatment. When the same eye score criteria were used but PCV $\leq$19 was used as a cutoff, sensitivity decreased to 92 and 94% for sheep and goats, respectively due to small numbers of false negatives (animals with PCV 16–19 that were scored as a 1 or a 2) (Tables 2, 6 and 10).

When eye scores of 4 and 5 were used as criteria for treatment, sensitivity decreased noticeably but there was a concurrent increase in specificity. Importantly, the number of false negatives also increased. Using a PCV cutoff of $\leq$19, the percentage of false negatives was 2.7 and 5.2% for sheep and goats, respectively (Tables 3 and 7). This level would not be considered acceptable under most management conditions. However, if the more reasonable cutoff of $\leq$15 was used, percentage of false negatives fell to 0.5 and 0.6% for sheep and goats, respectively (Tables 5 and 9). At this level, death from anemia would be a rare occurrence, especially if other common sense management and husbandry practices were used to identify and treat those animals at higher risk or who are displaying symptoms of clinical haemonchosis.

One might be tempted to view the relatively high rate of false positives using the FAMACHA© method as evidence that the test is not an accurate means for selecting animals for treatment. Such a conclusion would be a serious mistake. Using a traditional "treat all animals" approach and applying the same criteria for measuring false positivity would have resulted in a percentage of false positives in sheep of 92.4 (PCV $\leq$ 19) or 97.3% (PCV $\leq$ 15). In goats, the percentage of false positives would have been 87.7 (PCV $\leq$ 19) and 96.6% (PCV $\leq$ 15). Therefore, when compared to a "treat all animal" approach, the false positive rate using FAMACHA© in a selective treatment approach compares quite favorably.

Packed cell volume and FEC have been found to be correlated with the number of adult *H. contortus* worms present in sheep (Le Jambre et al., 1971). Therefore, as worm burdens with *H. contortus* increase, we expect to see a decrease in PCV, an increase in eye score, and an increase in FEC. This is precisely what was seen in this study; analyses of these data revealed highly significant correlations between PCV, eye score, and FEC. If all animals with eye scores of 3, 4 and 5 (for which FEC data were obtained) were treated, 50% of sheep and 63% of goats would have received anthelmintic. Assuming anthelmintic used was completely effective and FEC remained static, this level of treatment would have reduced mean FEC in the flock or herd by 71 and 83% respectively, for sheep and goats. If only animals with eye scores of 4 and 5 were treated, 14% of sheep and 31% of goats would have received anthelmintic. This level of treatment would have reduced mean FEC in the herd/flock by 35 and 46%, respectively for sheep and goats. These data reveal that treating all animals scoring 3, 4 and 5 will result in the administration of far more treatments than if only treating animals scoring 4 and 5, but the effect will be considerably greater reductions in pasture contamination with nematode eggs.

Ultimately, data from this study should form the basis of a set of guidelines for making treatment decisions when using FAMACHA© in the southern US. Since this system was developed and tested in South Africa, a brief review of the current recommendations for use of FAMACHA© in South Africa (J.A. van Wyk, personal communication) are warranted, before a set of recommendations are provided for the US based on the results of the current study. In the summer-rainfall area of South Africa, *Haemonchus* infection is seasonal. Following the dry winter period (June–August), a spring rise in FEC occurs due to both a resumption of transmission, and the development of hypobiotic worms to egg-laying adults. Translation of the parasite on pastures is slow during the spring, but as rainfall, temperatures and vegetative ground cover increase (conditions favorable for *Haemonchus* spp.) towards mid-summer (December), transmission of the parasite also occurs with increasing frequency. Parasite burdens tend to reach maximum levels in the late summer and early fall. In line with this seasonal trend, FAMACHA© examinations are carried out less frequently (e.g. every 3 weeks) during the spring and early summer, rising after good rains to weekly during the usually short peak in worm challenge. At the start of the worm season sheep must be treated when scored as 4 or 5, while animals scored as 3 are considered to be borderline. Sheep scored as 3 should, however, be treated when potential outbreaks of clinical haemonchosis are expected. Such periods of significant *Haemonchus* challenge appear to be heralded by a downward trend in the number of 1s and a reciprocal increase in 2s (Van

Wyk and Bath, 2002). Considerably less work has been carried out in goats than in sheep and given the apparent lesser accuracy of the FAMACHA© system in this species, it is recommended that animals scored as 3 always be treated. Along with these recommendations is always the warning that FAMACHA© should only be used in conjunction with a properly designed worm control program and when veterinary guidance is available.

Based on the results of this study for both sheep and goats in the southern US and the US Virgin Islands, it appears that treatment could be safely withheld until animals score as 4s or 5s as long as animals are in good body condition and good overall general health, are examined frequently (e.g. every 2 weeks) and good husbandry is used to identify animals in need of treatment (e.g. unthrifty, anorexic, lagging behind, bottle jaw) between FAMACHA© examinations. Using this approach, the number of anthelmintic treatments administered will be greatly reduced, resulting in diminished selection pressure for resistance and a concomitant reduction in drug costs. However, since animals need to be checked at frequent intervals, labor costs will be increased. Furthermore, it is recommended that this approach should only be applied to adult animals. Lambs and kids have comparatively small blood volumes and can progress rapidly from moderate to severe anemia. This precaution should also be extended to ewes and does extending from the periparturient through the lactation period, since these animals have decreased immunity to GIN (Courtney et al., 1984; Herd et al., 1983; Miller et al., 1998; Rahman and Collins, 1992). These and other animals that may be stressed by disease or poor body condition should always be treated if scored as 3s.

An alternative approach could be to treat all 3s, 4s and 5s. This will result in many more treatments being given to non-anemic animals, but will virtually eliminate the possibility that an anemic animal will fail to receive treatment. Also, because many animals scored as 3s still have high FEC, treating this group will greatly reduce egg contamination of pastures. Although many more treatments will be given, significant refugia will be maintained and the evolution of anthelmintic resistance should still be slowed considerably.

On farms where low to moderate levels of resistance has been diagnosed to one or more drugs (60–95% reduction in FEC), a useful strategy to help gain the full benefits of both treatment and resistance prevention could be to use these "less-effective" drugs either singly or in combination on all animals scored as 3s. Using drugs that are less effective in this group should not cause clinical problems to develop because the few 3s that are moderately anemic and in need of treatment, should receive a sufficient reprieve from infection until the next FAMACHA© examination, and the majority of the 3s which are not anemic do not need to be treated. This strategy will help preserve the efficacy of the drugs that are still fully effective by saving them only for the 4s and 5s, and also will help to minimize egg contamination of pastures.

Although FAMACHA© sounds easy to use, experience in South Africa and the southern United States suggests that proper training of farmers is required to effectively use this method. It is critical that users of FAMACHA© understand the risks of incorrect use of this system (e.g. animal mortalities) and necessary precautions that should be taken. Because animals are not treated until they become anemic, it is important that efficacy of anthelmintics is determined prior to using FAMACHA©. If anthelmintic treatments had been applied at frequent intervals prior to using FAMACHA© resistance may have been masked, especially if a rotation of drugs was used. In contrast, if treatment is withheld until animals are scored as 4s or 5s and a drug that has moderate to poor efficacy due to worm resistance is used, then deaths may occur. Other important precautions for using FAMACHA© include but are not limited to: the card is an aid in the control of *Haemonchus* spp. only; the system should be used by producers only where technical assistance is available from a veterinarian or other animal health professional; other management-based worm control practices must be maintained; smart drenching principles should be used (Hennessy, 1997; Mortensen et al., 2003); paleness or reddening of the conjunctivae may have other causes; animals should always be scored with the help of the chart, not from memory; examine animals at least every 2–3 weeks at the beginning of the expected period of *Haemonchus* challenge in climates where a seasonal incidence of infection occurs and during critical periods weekly examinations may be needed; protect the card from light when not in use; replace the card after 1 year of use (FAMACHA© Information Pamphlet).

In addition to the benefits of reducing drug costs and delaying the development of anthelmintic resistance, use of FAMACHA© can also help to improve the genetic resistance of individual herds or flocks (Bath et al., 2001). In the past several years, there have been several reports of successes in breeding lines of sheep that are genetically resistant to GIN. This was possible because host resistance to infection with *H. contortus* measured on the basis of FEC and PCV is a moderately heritable trait (Albers et al., 1987), and it has been demonstrated that the same animals tend to exhibit the highest FEC and lowest PCV on each occasion that they are measured (Barger and Dash, 1987). Importantly, data from recent investigations examining the heritability of resistance and resilience of Merino sheep to infection with *H. contortus* indicate a high heritability for the clinical estimates of FAMACHA© scores (Van Wyk and Bath, 2002). Since it can be expected that the same animals will require frequent treatments, and this trait of parasite susceptibility will be passed to the next generation, FAMACHA© can be a very useful tool for identifying animals to be culled. Removing the most susceptible animals from the breeding pool each year will have the long-term effect of improving the overall innate genetic resistance and/or resilience of the herd or flock to *H. contortus*. Such progress could never be made using traditional anthelmintic treatment approaches.

Results of the current study indicate that the FAMACHA© method is a very useful tool for identifying anemic sheep and goats in the southern US and US Virgin Islands. Data suggest that this method will make it possible for farmers to safely and reliably use a selective treatment approach for the control of *H. contortus*. Selective treatment used within a “smart drenching” framework together with novel non-chemical GIN control measures will reduce selection pressure for anthelmintic resistance and maintain the efficacy of anthelmintics into the future. However, insufficient data and experience exist to know precisely what will be the best strategies for applying this system. Consistent with recommendations in South Africa, FAMACHA© should only be used when veterinary guidance is available. Differences between farms in overall quality of management, stocking rates, breeds of animals, pre-existing levels and spectrum of anthelmintic resistance, presence of nematode species other than *H. contortus* and production targets, will all have an important impact on how best to manage parasites. Including FAMACHA© as part of an integrated control program does not change this. General guidelines for integrating FAMACHA© have been presented, but as is the case with any well-designed parasite control program, optimal strategies need to be tailored to meet the needs of individual farms.

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